Pergamon

Tetrahedron Letters 41 (2000) 61–64

TETRAHEDRON LETTERS

# Structural elucidation of new antibiotic peptides, atroviridins A, B and C from *Trichoderma atroviride*

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Received 14 September 1999; revised 13 October 1999; accepted 18 October 1999

**Abstract**

Three peptaibols, atroviridins A–C, were isolated from the culture broth of *Trichoderma atroviride*. The amino acid sequences were determined by mass spectrometry and two-dimensional NMR experiments. They are composed of 20 residues with a high ratio of α-aminoisobutyric acid, and these all are determined as new peptaibols. © 1999 Elsevier Science Ltd. All rights reserved.

Peptaibols are a class of linear antibiotic peptides biosynthesized by widespread soil fungi, such as *Trichoderma*, *Hypocrea*, *Emericellopsis* and *Boletus* genus.[1] Structurally, peptides of this class are charaterized by several α,α-dialkyl amino acid residues such as α-aminoisobutyric acid (Aib), an N-terminal acyl group, and the presence of a C-terminal amino alcohol.[2] Because of the relatively high content of Aib, the strongest known helix-forming amino acid,[3] peptaibols have a strong tendency to form amphipathic helices resulting in voltage-dependent transmembrane channels in the lipid bilayer membrane.[4] Some of them were shown to induce various biological activities, such as uncoupling of oxidative phosphorylation in mitochondria,[5] enhancement of $Ca^{2+}$-dependent catecholamine release from adrenal chromaffin cells,[6] hemolysis,[7] cell fusion,[8] and inhibition of ameba cell multiplication.[9]

In this paper, we report the stuctural elucidation of new peptaibols, atoviridins A–C isolated from the liquid culture of *Trichoderma atroviride* (Table 1).[10] This is the first report isolating peptaibols from *T. atroviride*.

Their sequences were determined by positive ion FAB-MS and two dimensional NMR experiments (COSY, HMQC, HMBC, TOCSY, ROESY). Amino acid analysis of the total acidic hydrolysates of atroviridin A provided the following amino acid composition: Aib (8), Ala (2), Glx (3), Gly (1), Val (2), Leu (1), Pro (2). Atoviridin B differs from atroviridin A in that it contains isovaline (Iva, α-amino-α-methyl butyric acid) (1) instead of Aib (1) and Ala (1) in atroviridin B is changed to Aib (1) in atroviridin C.

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*PII:* S0040-4039(99)02000-6

Table 1
Sequences of atroviridins A–C

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Ac-Aib-Pro-Aib-Ala-Aib-**Ala**-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val– | | | | | | | | | | | | | | | Aib-**Aib**-Gln-Gln-Pheol | | | | |
| **B** | Ac-Aib-Pro-Aib-Ala-Aib-**Ala**-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val– | | | | | | | | | | | | | | | Aib-**Iva**-Gln-Gln-Pheol | | | | |
| **C** | Ac-Aib-Pro-Aib-Ala-Aib-**Aib**-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val– | | | | | | | | | | | | | | | Aib-**Iva**-Gln-Gln-Pheol | | | | |

The three Glx residues obtained in the total acid hydrolysates were assigned to Gln from the observation of the *syn* and *anti* ε-protons of the three carboxamide groups in the $^{1}$H NMR and TOCSY spectra. To determine the absolute configuration of each amino acid, GC analysis of the total hydrolysates with derivatization was employed. GC analytical conditions were the same as described previously.[11] The GC analysis results indicated that the chirality of all the amino acids and the amino alcohol, phenylalaninol (Pheol, Fol), were in L, except for isovaline which was in D.

The FAB-mass spectrum of a major component, atroviridin A, gave an $[M+Na]^{+}$ ion with an *m/z* 1986. Also two major fragment ions are obtained at *m/z* 1189 and 774, which were generated from the Aib–Pro bond with a labile tertiary amide link. The abundant fragmentation ion at *m/z* 1189 turned out to correspond to the N-terminal part of atroviridin A, and the fragmentation ion at *m/z* 774 corresponds to the C-terminal part. The collision-induced dissociation (CID) spectrum of the ion at 1189 showed successive acylium ions assignable as shown in Fig. 1a. It provided sequence-specific ions at *m/z* 1104, 991, 934, 849, 750, 537, 466, 381, 310, 225, and 154 and the mass differences of these sequence-specific ions are attributable to Aib, Leu, Gly, Aib, Val, Gln+Aib, Ala, Aib, Ala, Aib, Pro, Ac-Aib for the sequence

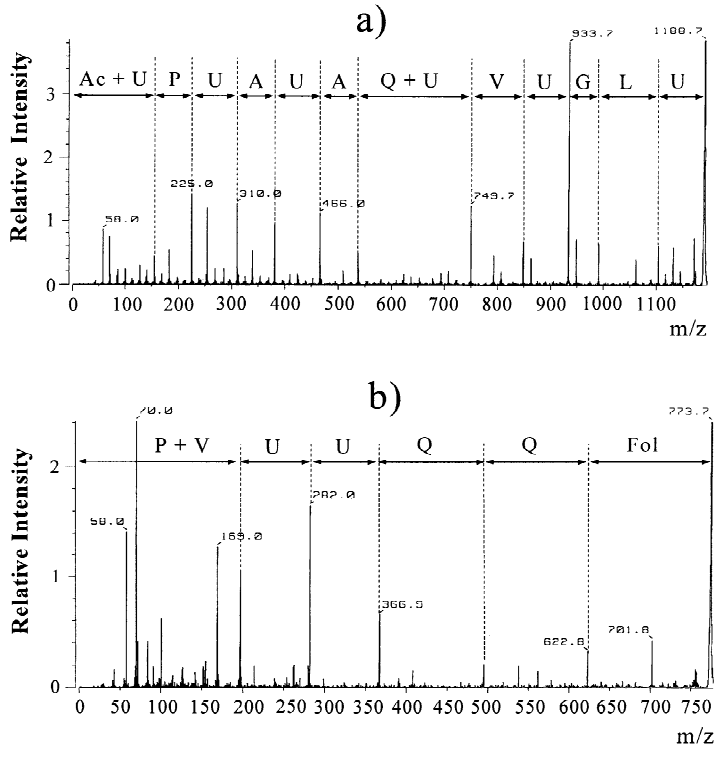


Figure 1. CID spectrum of the ions *m/z* 1189 (a) and 774 (b) from atroviridin A (U=Aib, Fol=Pheol)

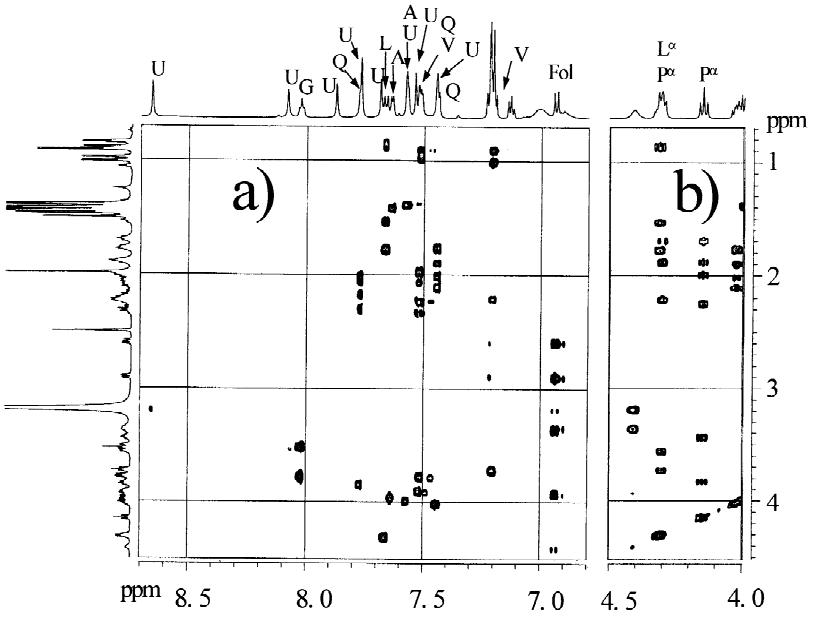


Figure 2. NH (a) and ProC$^{\alpha}$H (b) regions of TOCSY spectrum of atroviridin A in DMSO-$d_6$ at 60°C

1–13, respectively. However, due to absence of the sequence-specific ion between *m/z* 750 and 537 in CID spectrum, the sequence 7–8 could not be determined. The CID spectrum of the peak at *m/z* 774 afforded sequence-specific ions except for Pro+Val of C-terminal residues as shown in Fig. 1b. However, the spectrum showed the significantly abundant B-type ion (*m/z* 70) that was deduced to originate from the loss of CO for Pro. Thus, the sequence of C-terminal residues was determined as Pro-Val-Aib-Aib-Gln-Gln-Pheol.

The proton chemical shifts of each amino acid moiety were assigned by TOCSY spectrum. Eighteen amide protons except for two prolines were assigned at the region of δ 6.9~8.7. Each C$^{\alpha}$H of two prolines was observed at δ 4.32 and 4.15, respectively (Fig. 2).

Amino acids sequences of 7–8 that still remained uncertain were determined by ROESY experiment. The ROESY spectrum gave the conclusive evidence for the amino acid sequence of 7–8, which showed the correlation peaks of NH(Ala$^{6}$)/NH(Glu$^{7}$), NH(Glu$^{7}$)/NH(Aib$^{8}$), and NH(Aib$^{8}$)/NH(Val$^{9}$) in Fig. 3. This also confirmed the sequence of C$^{\alpha}$H(Pro$^{14}$)/NH(Val$^{15}$). Thus, the structure of atroviridin A was concluded to be Ac-Aib$^{1}$-Pro$^{2}$-Aib$^{3}$-Ala$^{4}$-Aib$^{5}$-Ala$^{6}$-Gln$^{7}$-Aib$^{8}$-Val$^{9}$-Aib$^{10}$-Gly$^{11}$-Leu$^{12}$-Aib$^{13}$-Pro$^{14}$-Val$^{15}$-Aib$^{16}$-Aib$^{17}$-Gln$^{18}$-Gln$^{19}$-Pheol$^{20}$. The sequence of atroviridins B and C was determined by the combination of FAB-MS and two dimensional NMR experiments on the basis of the sequence of atroviridin A.

**Acknowledgements**

The authors thank Ms. Eun-Hee Kim and Mr. Young-Hwan Kim (Korea Basic Science Institue, Taejon) for skillful records of 600 MHz two dimensional NMR analysis and tandem mass spectrum. We are also grateful to Mr. Kyu-Hwan Park for FAB-MS experiments (Korea Research Institute of Bioscience and Biotechnology, Taejon).

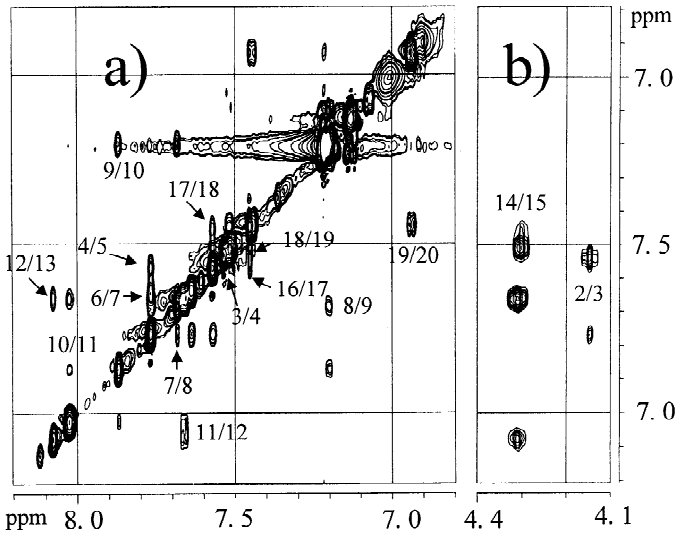


Figure 3. NH–NH (a) and Pro$C^{\alpha}$H–NH (b) cross peaks of the ROESY spectrum of atroviridin A

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